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# ERRORS IN AUTOMATED CELL COUNTER: MAJOR PITFALL WITH RISK OF MISINTERPRETATION OF RESULTS

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***ABSTRACT***

***Introduction***

***Currently available automated cell counters are capable of analyzing various parameters of complete blood count efficiently. We report 2 cases of hematological investigations which helped us to understand errors in automated cell counter readings which caused results misinterpretation.***

***Material and Methods***

***A 27years/Female presented with headache and weakness for 4months. Hemoglobin was12.6g/dl, total leucocyte count22.9X10$^9$/L, platelets271X10$^9$/L and reported as acute leukemia with 46%blasts. On day of bone marrow examination, peripheral blood findings did not correlate with previous findings. Bone marrow was normocellular (M:E ratio 3:1) with no evidence of leukemia/lymphoma. So all haemogram reports were reviewed which were done on same day when this sample was first received.***

***Results***

***Peripheral blood findings were found to be similar to those of a prior sample. Sampling/technical errors were ruled out and we concluded that automated cell counter might not be effective in autorinsing function. So some cells might have been transferred to next sample.***

***Conclusions***

***The erroneous result was due to error in autorinsing by the automated cell counter and due to improper wiping off of spreader while preparing peripheral blood films. Hence at every step, utmost care must be taken starting from sample collection, sample labeling, requisition form, sample processing, smear preparation, staining and other investigations.***

***KEYWORDS: Automated Cell Counter, Error, leukemia/Lymphoma, Misinterpretation & Haemogram***

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Original Article

## INTRODUCTION

Complete blood count is the most commonly performed test in a clinical hematology laboratory. Currently available automated hematology cell counters are capable of analyzing the various parameters of complete blood count fairly reliably and efficiently. Peripheral blood film is usually examined for few cases only when automated cell counter displays flags. However, a microscopic examination of an appropriately prepared and

well-stained blood smear is necessary which is clinically useful in number of diagnosis.[1,2] We report 2 cases of hematological investigations which helped us to understand the pitfalls in interpretation of results due to errors in automated cell counter readings as well as errors while preparing peripheral smear.

## MATERIAL AND METHODS

We report a case of 27 years female who presented with headache, weakness and dizziness for last 4 months. On physical examination, there was no pallor/ significant organomegaly/ lymphadenopathy. In routine laboratory findings, her complete blood count was done which showed hemoglobin- 12.6 g/dl, total leucocyte count i.e. TLC- $22.9X10^9$/L, differential leucocyte count i.e. DLC- blasts 46, neutrophils 19, lymphocytes 27, monocytes 3, eosinophils 3 myelocytes 2, metamyelocytes 2, platelets- $271X10^9$/L (Table 1). Blasts were large cells with high N:C ratio, fine nuclear chromatin, prominent 1-2 nucleoli and moderate amount of granular cytoplasm. Final report was given as acute leukemia with 46% blasts and bone marrow examination was further advised (Figure 1).

## RESULTS

On the day of bone marrow examination, the complete blood count was done again which showed hemoglobin- 12.8g/dl, total leucocyte count i.e. TLC- $10.6X10^9$/L, differential leucocyte count i.e. DLC- neutrophils 58, lymphocytes 39, monocytes 2, eosinophils 1, platelets- $275X10^9$/L. These did not correlate with the findings which were of previous sample from the same patient.

The bone marrow examination showed normocellular marrow aspirate with myeloid to erythroid ratio 3:1. The erythroid series was normoblastic. Myelogram was blasts 4%, promyelocyte 1%, myelocytes 12%, metamyelocytes 8%, neutrophils 32%, lymphocytes 15%, monocytes 1%, eosinophils 1%, plasma cells 2% and erythroid cells 24%. Megakaryocytes were adequate. Bilateral trephine biospsies showed normocellular marrow spaces and all the three hematopoietic series cells i.e. erythroid, myeloid and megakaryocytic were adequately represented. Reticulin stain showed normal fibrosis, WHO grade 1 (Table 2). There was no evidence of leukemia/ lymphoma (Figure 2).

All the haemogram reports were reviewed which were done on the same day when this sample was first received in the routine hematology laboratory. The complete blood count of this patient's sample was reviewed and cross checked to look out for any miss matching or sampling/ technical errors during routine laboratory processing when we received patient's sample for the first time. We found that a sample which was received prior to the sample of this patient was reported out with similar findings of acute leukemia. The complete blood count findings of the previous patient's sample were- haemoglobin- 4.6 g/dl, TLC- $278.6X10^9$/L, differential leucocyte count i.e. DLC- blasts 87, neutrophils 7, lymphocytes 4, monocytes 1, myelocyte 1, metamyelocyte 1, platelets- $19.0X10^9$/L. Blasts were large cells with high N:C ratio, fine nuclear chromatin, prominent 1-2 nucleoli and moderate amount of granular cytoplasm. Final report was given as acute leukemia with 87% blasts and bone marrow examination was further advised (Figure 3). However this patient was very poor and not affording so left against medical advice.

## DISCUSSIONS

Both the samples were received and numbered in consecutive serial order in our routine hematology laboratory (Table 3). The complete blood count findings and peripheral smears of samples from both the patient's were cross-checked. Errors at various phases i.e. pre-analytical, analytical and post-analytical phase were checked. It has been

estimated that up to 62% errors happen during pre-analytical phases.[3] The pre-analytical errors such as patient's misidentification, requisition form details incorrect or inadequate (name, age, clinical history, investigations to be done, provisional diagnosis), patient's sample mislabelling, incorrect samples taken, wrong choice of vials, diluted samples, hemolysed samples, prolonged time gap between sample withdrawn and sample sent, sample storage, sample transport and sample receiving were cross-checked. The analytical errors include matching of samples label with that of the smears prepared, tests performed on unsuitable sample, the spreader being used, error at the level of automated cell counter. Various errors related to the automated cell counter use itself are there like standardization of the instrument not done by running quality controls everyday to check for accuracy of the instrument, linearity check, precision of the instrument not checked by analyzing the data run on replicate testing.[4] In case of very low or high count in the sample, flags are usually displayed on screen of the automated hematology cell analyzers. Autorinsing is another step where the error can occur if this function is inefficient. Controls were run daily to standardize the automated cell counter in our laboratory. Also the post-analytical errors were checked which included error during dispatching of the report/ wrong reporting destination. Root cause analysis was done and after ruling out before mentioned errors, we were left only with technical errors.[5-7]

We found error during the analytical phase. The automated cell counter has the function of autorinsing i.e. the probe which is used for sucking the blood sample into the machine is autorinsed by the cell counter itself after each sample being sucked in for data interpretation. This function may not be effective at some point which could be one of the reasons for the data misinterpretation by the automated cell counter. Due to high TLC in the first sample, some cells might have been transferred to the next sample leading to erroneous readings in the next sample.

Another error was found to be due to the use of spreader. For all the peripheral smears being prepared in our routine laboratory, the spreader used is same. After preparing a peripheral blood film, the spreader is wiped off of blood by a guaze piece/ a tissue paper and then being used for preparing next sample's peripheral smear. The improper wiping off of blood from the spreader might have led to transfer of cells from first sample to the next one.

## CONCLUSIONS- LESSONS LEARNT

Complete blood count and the peripheral smear examination are the basic and highly informative tools in screening and diagnosis of a disease. Automated cell counter is routinely used in screening of all the blood samples. The peripheral smear acts as quality control in verifying the results generated by automated cell counter. There was an error in proper washing (autorinsing of the probe) after one sample was sucked in by the automated cell counter for analysis. Also, the spreader used was common for all the samples which led to transfer of tumour cells to the next patient's peripheral smear. So at every step, utmost care must be taken starting from sample collection, sample labeling, requisition form, sample processing, smear preparation, staining and other investigations. Keeping track of the errors may be helpful in reducing the errors occurring during later processes.

Besides these, monthly workshops or CMEs and proficiency testing can be planned to train the nurses, technical staffs and even doctors about the pre-analytical, analytical and post-analytical phases of error, about various parameters, standardization and quality control of the laboratory manual or automated instruments and their importance. Quality manuals should be available to each and every staff to further reduce the chances of errors.

## CONFLICTS OF INTERESTS: Nil

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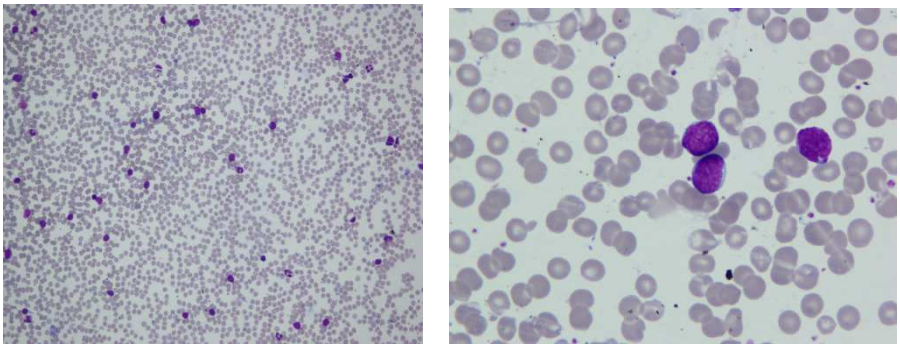
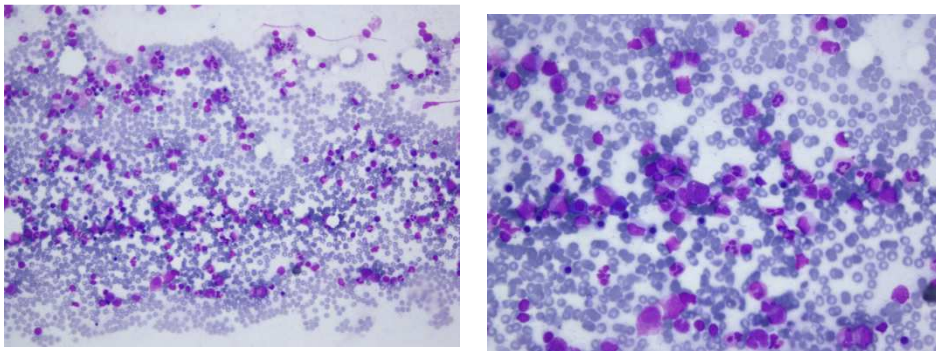
## APPENDICES

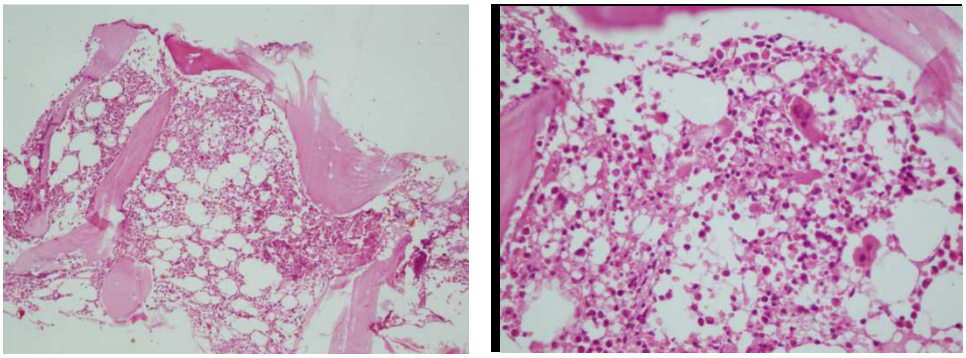


**A: 100X, Leishmann Stain; B: Oil Immersion, Leishmann Stain**

**Figure 1: Peripheral Blood Smears Shows Raised TLC with Presence of Blasts**

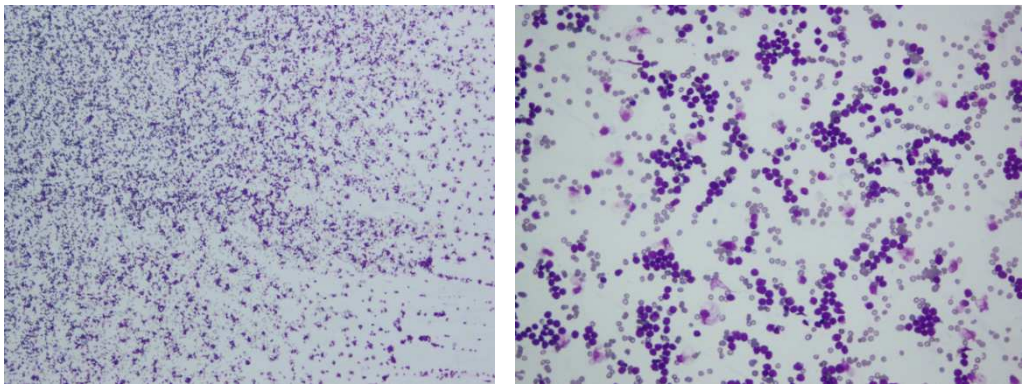


**A: 20X, MGG Stain; B: 40X, MGG Stain**

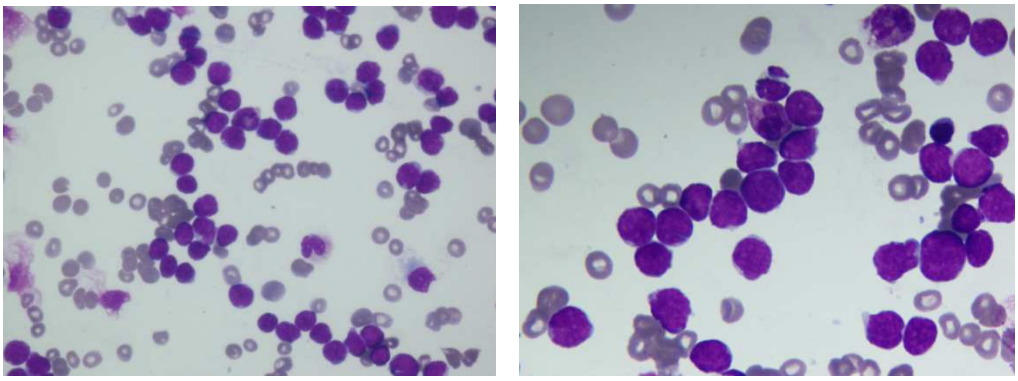


**C: 100X, H&E Stain;** **D: 400X, H&E Stain**

**Figure 2: Bone Marrow Aspirate Showed No Evidence of Any Leukemia/Lymphoma**



**A: 40X, Leishmann Stain,** **B: 200X, Leishmann Stain**



**C: 600X, Leishmann Stain,** **D: Oil Immersion, Leishmann Stain**

**Figure 3: Peripheral Smear Findings of the Patient's Sample which was Originally Reported as Acute Leukemia**

**Table 1: Laboratory Investigations: Hemogram Findings**

| Investigations | Results |
|---|---|
| **Hemoglobin** | 12.6 g/dl |
| **Total leucocyte count** | $22.9X10^9/L$ |
| **Differential leucocyte count** | Blasts 46, neutrophils 19, lymphocytes 27, monocytes 3, eosinophils 3 myelocytes 2, metamyelocytes 2 |
| **Reticulocyte count** | 3.0% |
| **Platelets** | $271X10^9/L$ |
| **Red blood cell indices** | MCV: 84fl, MCH: 27pg, MCHC: 33% |

**Table 2: Laboratory Investigations: Bone Marrow Examination**

| Investigations | Results |
|---|---|
| **BONE MARROW ASPIRATE** | |
| **Cellularity** | Normocellular |
| **Myeloid to erythroid ratio** | 3:1 |
| **Erythroid series** | Normoblastic |
| **Myelogram** | Blasts 4%, promyelocyte 1%, myelocytes 12%, metamyelocytes 8%, neutrophils 32%, lymphocytes 15%, monocytes 1%, eosinophils 1%, plasma cells 2% and erythroid cells 24% |
| **Megakaryocytes** | Adequate |
| **BONE MARROW TREPHINE BIOPSY** | |
| **Cellularity** | 50-55% |
| **Erythroid series** | Adequately represented |
| **Myeloid series** | Adequately represented |
| **Megakaryocytic series** | Adequately represented |
| **Reticulin** | Normal (WHO Grade 1) |

**Table 3: Laboratory Investigations: Comparison of Hemogram Findings of the 2 Patient's Samples which were Received on the Same Day**

| Investigations | Results of Patient 1 (Case Study) | Results of Patient 2 |
|---|---|---|
| **Hemoglobin** | 12.6 g/dl | 4.6 g/dl |
| **Total leucocyte count** | $22.9X10^9/L$ | $2,78.6\ X10^9/L$ |
| **Differential leucocyte count** | Blasts 46, neutrophils 19, lymphocytes 27, monocytes 3, eosinophils 3 myelocytes 2, metamyelocytes 2 | Blasts 87, neutrophils 07, lymphocytes 04, monocytes 1, myelocytes 1, metamyelocytes 1 |
| **Reticulocyte count** | 3.0% | 0.1% |
| **Platelets** | $271\ X10^9/L$ | $19.0\ X10^9/L$ |
| **Red blood cell indices** | MCV: 85fl, MCH: 28pg, MCHC: 33% | MCV: 94fl, MCH: 44pg, MCHC: 47% |